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YUGOSLAV INSTITUTE OF MEAT TECHNOLOGY
Beograd, Kaćanskog 13

A STUDY OF THE CONTRIBUTION OF BRINE MICROORGANISMS AND THEIR MUTANTS TO THE MEAT CURING PROCESS, TO OBTAIN INFORMATION APLICABLE TO THE PREPARATION OF IMPROVED CURED MEAT PRODUCTS

Beograd, 1973.

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S U M M A R Y

"Salting" is an important phase in the production of cured meat pieces. The final results of microbiological, phusic- and biochemical changes are specific colour, taste and odour - properties which classify these products in the group of "delicious" products. Curing processes of meat in pieces are as a rule, of long duration and therefore uneconomical for industrial production.

As a result of the carried out examinations so far, the time of curing processes has been shortened in relation to previous curing processes. Products prepared from meat in pieces (dry ham, dry loin etc.) and produced by a curing process of "shorter" duration are without specific taste and odour due to which they are appreciated by consumers. The reason why processes of "shorter" duration could not replace those of "longer duration" was ignorance of changes and compounds appearing during curing processes and being the carriers of taste and odour of dry ham, neck, loin, bacon and similar dried cured meat product. In addition, mutual influences of the activity of microorganism enzymes and that of meat enzymes, during long-term curing processes, were not known.

The aim of these examinations was to explain the role of microorganisms in curing processes and to examine the possibility of applying the obtained results in the practice.

The most important results obtained by these examinations are as follows:

- Microorganisms take part in the changes of meat and fatty tissue during long-term dry curing processes. Dependent on the size of pieces, conditions and duration of curing, the role of microorganisms is smaller or bigger and they affect deep interior layers or only surface layers, as is the case in dry cured hams.

- Microorganisms isolated by a special, original technique, can be applied in the practice with the purpose of accelerating the production of cured, dried meat pieces having excellent taste and odour, colour and consistency.

- Prior to their use as "starter" cultures, microorganisms isolated and selected within these examinations must be grown under special conditions and in a medium rich in sugar.

"Bundiolla" and dry bacon of excellent organolentical properties were produced by the process of "shorter" duration, using the selected microorganisms isolated as dominant microflora during dry curing of "bundiolla" and bacon and grown by appropriate technique of growth at low temperature and in the presence of sugar. The results of our examinations confirm that "burdiolla" and dry bacon can be produced by the process of "shorter" duration in industrial conditions as well. Due

to the lack of time, it was not possible to carry out similar examinations with larger pieces of meat, such as "pršut". Therefore it is not known whether the production process of "pršut" could be shortened by eventual introduction of substrates, enriched with the matters resulting from biochemical activity of microorganisms and with the selected microorganisms themselves, into deep interior layers of ham muscles, on condition that "pršut" keeps or even improves its organoleptical characteristics. In our opinion, continuation of examinations in that direction is economically justifiable because "pršut" is a highly appreciated product at all world markets. Up to present, "pršut" has not been industrially produced anywhere. Our presumption based on theoretical considerations that "pršut" can be industrially produced should be experimentally proved.

D E T A I L E D R E P O R T

INTRODUCTION

Curing - a complex physico- and biochemical process in which the microorganisms also take part - is well known in the manufacture of ground meat products. Factors taking part in this complex mechanism are explained in the literature in details. However, this is not the case in the process of dry or dry-wet curing of large meat pieces. The changes appearing in meat and fatty tissue of loin, neck, ham and bacon, during their long-term dry salting or curing are, specially insufficiently explained in the science and practice. Namely, processes and compounds taking part in the formation of specific and therefore exceptionally appreciated taste and odour of these products have not been defined as yet. Although in the literature there is a large number of works on microorganisms and their role in long-term curing processes, their real significance from the standpoint of science and even more from the standpoint of their application in practice has not been defined so far. Their effect on the changes of meat and on the formation of compounds taking part in the appearance of "aroma of cured ham of "pršut" type or cured neck of "bundiolla" type is still less known. This is not peculiar because complexes of compounds - carriers of taste and odour of dry cured meat products are still unknown in the science, in spite of numerous investigations the results of which are

available in the literature. It is still disputable in the literature whether the appearance of aroma carriers is due to the change of muscular tissue or that of fatty tissues, or of both tissues simultaneously, during curing and "ageing" of dry hams and similar products. Contradictory opinions, as well as examinations of similar products being often produced by different procedures in various countries, bring confusion in this field of science. The problem connected with brine microflora and meat microflora is specially complex. Some schools recommend different examination techniques and even more they enable comprehension of the problem as a whole because of different interpretation of the results. There is not an agreed opinion on microflora being dominant in the curing process and so it is quite understandable that even the role of microflora in the changes appearing in meat during its curing cannot be defined. It can be presumed that there has not been enough explanations up-to-now because the examinations were one-sided and the mechanism of the appearance of taste and odour of meat products is complex to such an extent that the proper answer can be obtained only by comprehensive examinations. On the basis of the literature data, it is known that there are more compounds appearing during the changes of muscular and fatty tissues and for which it has been established that they contribute to the formation of meat product aroma. A large number of authors, including Ambanelli, Batzler,

Santora, Landman, Bianchi, Colombo, Gervasini, Eddy, Crau, Körmendy, Lubanov, MacCain, Macy and others; established changes in the content and kind of amino acids, amines and other nitrogen compounds during curing and aging of meat, emphasizing their definite significance in the aroma formation.

Examinations of fat changes were also performed by a large number of authors. On the basis of the obtained results, Bahonina, Bianchi, Boljšakov, Cantoni, Eser, Farkaš, Giolitti, Garović-Vlaseva, MacCain, OcKerman, Blumer and Craig state, probably rightfully, that carbonyl compounds appearing in meat products represent even the main carriers of their aroma.

According to some authors, the content, namely the composition of free and specially volatile fatty acids can also be of significance for the appearance of taste and odour of certain meat products (Cantoni, Molnar, Renon; Giolitti; Kamis; Otaiski; Langner; Shnak and Silliker).

On the basis of their own examination results, Cantoni, Molnar, Renon and Giolitti, then Macy, Nauman, Bailey and Milton, concluded that, in some meat products, phosphatides appearing during the production process should be classified as carriers of taste and odour. A small number of authors (Batzler, Santoro, Tor and Landman; Macy, Nauman, Bailey and Milton) indicate to significant changes in the content and kind of

sugars during the production process of meat products, supporting this statement with enough arguments. On the basis of this statement, they present the presumption that sugars also take part in the changes manifested as specific aroma of meat products.

It has been stated that sulphur-bearing compounds, mercaptan and hydrogen sulphide can also be the participants of the complex rendering taste and odour to some meat products (Zoltowska; Hornstein, Crowe, Sulzbacher, Barnett, Nordin, Bird and Rubin).

In addition to already mentioned compounds for which it has been proved or presumed by various authors that they take part in the formation of taste and odour of meat and meat products, Solovjev; Hermann; and Böhm are of opinion that the significance of nucleotides, nucleosides, purines, as well as their products of decomposition, should not be neglected when the meat aroma is considered.

The role of nitrates and nitrites in the formation of cured meat colour has already been explained. However, according to the opinion of a large number of authors, they also take part in the formation of specific taste of cured meat, through the reaction occuring between nitrites and some components of muscular tissue (Brooks, Haines, Moran and Pace; Gross, Leigler and Rubin; Eddy; Grau and Böhm; and Palitzsch).

The literature data on the microflora of brine and cured meat could be divided into several basic fields.

Many authors present the data on the count and kind of bacteria and other microorganisms, isolated from curing brine or meat products. Pallu; Džapo; Džinleski; Graham and Blumer; Giolitti et al.; Stöjanov; Verbelen; Cantoni et al., as well as many other authors, only state the presence of microorganisms during the curing process, what is quite logical because they examined various curing brines for pumping or covering, as well as different parts of carcasses deriving from different kinds of animals. However, they did not give a clear answer regarding the count of microorganisms in different stages of curing, as well as regarding the mutual relation of individual bacteria kinds isolated during the production of different kinds of cured meat products.

As for the finding and significance of individual bacteria kinds and microorganisms in general, and specially when determination of microflora during curing process is in question, one hardly can get along with the mass of different and often diametrically opposite results. An exceptionally large number of authors considered this problem (Sulzbacher; Pallu; Annamaria Ferrari et al.; Kuharkova et al.; Leistner; Palamarov; Graham and Blumer; Giolitti et al.). The only conclusion that can be drawn out on the basis of their examination results is

that different microflora appears during the curing process of different meat products. Dependent on a large number of factors, such as concentrations of salt, nitrates, nitrites and sugar, pH value, temperature and time, halotolerant Gram--positive or Gram-negative microorganisms adapted to psychrophilic conditions dominate during the curing process.

Dependent on the group to which they belong, dominant microorganisms express different enzymic activity. Among other things, some of them are capable of forming desirable or undesirable odour when grown on specific media, as established by Glago; Rašeta; Kuhole; Heiko and others. However, it is still unknown whether they can manifest this property in products from which they were isolated, but in which they are very often present only in a small number.

Up-to-now obtained knowledge on the tolerance of microorganisms to salts is significant, although the preservation effect of salts on microorganisms in meat and meat products has not yet been defined. However, the first prerequisite for microorganisms to act positively during curing is undoubtedly their tolerance to salt to a certain extent. More precisely said, they must grow and express biochemical activity in media containing a relatively high content of NaCl. Precious data within this field were obtained by numerous authors (Jensen; Niinivaara; Volifero; Savić, Mailet; Rockvel et al.; Pettersen; Aleiev;

Fischer; Ingram and Kitchell; Vojtkevič; Graham; Jons; Calow; Kuharkova; Pohja; Pallu and many others).

We should agree with the opinion of Wolnak according to which there still exists a sophistic look at the application of enzymes of microbiological origin in the production of foods. The reason should be looked for in the fact that the sphere of useful enzymic action has not been sufficiently studied up as yet. There is specially a lack in the data referring to biochemical activity of microorganisms grown on natural substrates, such as meat and fatty tissue. Very useful data covering this field have recently been obtained by Halmann, Smith and Alford; Reuter, Borton, Urbanik, and a group of Italian authors - Cantoni, Giolitti, Biank and others.

As a result of all achievements within this field, in the literature there appear numerous data regarding the attempts of using "active" bacterial strains in the practice. Although the problem as a whole is far from the complete solution, numerous patents appeared, specially during last years. The patents serve to authors and manufacturers to protect the freez-dried (or preserved in some other way) "active" - individual or mixed - cultures. The preparations are advertised to a high extent, although their real value has not been completely proved and probably because of that their use in practice has not reached larger proportions up to present.

On the basis of the literature data (Kuharkova; Mihajlova, Bothast, Schiffner and Oppel; Paljmin and Genockoj; Dičevi-niv, Wighlacz and Wyslauch; Esko Petäja; Niinivaara, Nurmi, Pohja, Č. Tadić), it can be seen that attempts are made in all countries to obtain meat products of higher quality by applying bacterial cultures, or to use bacterial cultures for the preparation of products by industrial technology. Recent data speak in the favour of the thesis that the application of metabolitic products is more adequate for the solution of this problem, than the application of microorganisms themselves.

Having in mind all up to now established facts, the main aim of this work was to examine (during dry-curing of hams and necks for the manufacture of "pršut" and "bundiola"):

- Mutual relation of microorganism counts during different stages of the curing process;
- Microflora being dominant during dry-curing process;
- Biochemical activity of microflora being dominant during dry--curing process, at temperatures ranging from 0 to 4°C; and
- Possibilities of improving organoleptical properties of dry--cured products, using selected cultures and their combinations.

RESULTS

On purpose to answer the questions set by this project, and applying the technique described in details in the last two annual reports, the examinations of pork neck (for "bundiolla"), fatty tissue (for dry bacon) and ham (for "pršut"), during their curing were performed.

For better clearness, the obtained results - the same as in previous reports - are divided into three parts:

A. Examinations of pork neck
B. Examinations of bacon
C. Examinations of ham

A. Examinations of pork neck

These examinations included the following:

1. Chemical and physico-chemical changes of neck during its curing;

2. Orientation examination of active microflora in neck during its curing;

3. Group identification of microflora being dominant in neck during its curing;

4. Examination of the catalase reaction of isolated Gram-positive cocci and bacilli;

5. Examination of more important biochemical properties of microflora being dominant in neck during its curing;

6. Examination of the activity of selected microorganisms (being dominant in neck during its curing) on fatty tissue;

7. Experimental checking of the growth and activity of selected microorganisms in cured and dried muscular tissue; and

8. Experimental production of dry pork neck by applying the selected culture combinations.

The examination results presented in previous reports (1 through 7) indicate that some selected cultures, and even more certain combinations of selected cultures, influence organoleptical properties of cured meat. Encouraging results were also obtained on the medium prepared from fatty tissue. Starting from these data, "burdiolla" and "dry bacon" were experimentally produced by applying the selected culture combinations. The results of these examinations are presented in this report.

Experimental production of dry pork neck by applying selected culture combinations

Cooled pork neck, prepared in the usual way, were injected with selected culture combinations (A, B, C and D), dry-cured in production conditions and then dried in the "Travaglini" clima chamber at 12 - 15°C and at relative humidity of 70-80%, for 36 days.

The necks, weighing 1500 g. on average, were cured with dry curing mixture containing 95% of common salt, 1% of $NaNO_3$ and 4% of sugar. Cultures of the following strains; 04 (var. Lactobacillus plantarum), 018 (micrococcus 18), 037 (Lactobacillus plantarum) and 059 (yeast being still undetermined in details), were prepared by a special, previously described technique. The

selected combinations were obtained by mixing the suspensions of selected cultures in 1:1 ratio, as follows: A-04 and 037, B-018 and 037, C-018 and 059, and D-018 and 04. After homogenization performed by vibration, the selected culture combinations were injected, in 1.5% amounts, into "salted" necks, by means of long, perforated needles. The control group was injected with the corresponding quantity of 0.5% sugar solution.

1. Bacteriological examination of pork neck injected with selected culture combinations.- The results obtained by bacteriological examination of neck after its curing and after drying for 20 and 36 days, are presented in Figure 1.

After the completion of curing, total bacteria count, as well as individual counts of sacharolytic and lipolytic bacteria, in all samples injected with selected culture combinations, ranged mainly within the same limits, both on the surface and in deep interior layers of the neck meat, being about 10^6 - 10^7 in 1 g. on average. The only exception was a considerably lower count of lipolytic bacteria in samples injected with the culture combination A. As it could be expected, the control samples showed a considerably lower bacteria count, specially in deep interior layers.

After drying for 20 days, total bacteria count, as well as their lipolytic and saccharolytic activities, remained more or less the same as in the previous stage.

After the completion of drying, namely in finished products, total count of bacteria and the count of saccharolytic bacteria were only slightly reduced or equal to values obtained in previous stages; however, the count of lipolytic microorganisms was more or less reduced in all cases.

2. *Some chemical examinations of pork neck injected with selected culture combinations.* - Tables 1, 2 and 3 present the results of some chemical examinations performed after curing and during drying of neck samples injected with selected culture combinations.

After the completion of curing and after 20 and 36 days of drying, the content of water shows a constant decrease in all cases. Contrary to that, the contents of fat and protein are increased proportionally to the quantity of lost water. The percentage of NaCl also shows a natural increase and in finished products it ranges between 5 and 6.7%; similar statement can be set for the content of nitrites, whereas the content of nitrates shows a reverse trend. The pH value of the neck muscular tissue also shows a normally expected decrease. Peroxide value of fat extracted from neck samples is increased from stage to stage and at the end of drying it is over 4. The content of free fatty acids is increased, specially at the end of drying, in all injected samples, but not in the controls.

Tables 4a, 4b and 4c present changes in the content of free amino acids after curing (4a), after drying for 20 days (4b) and after drying for 36 days (4c), of experimentally produced "bundiolla". Sudden increase of the content of free amino acids is observed in all examined samples after curing for 12 days and after drying for 20 days (Tables 4a and 4b). A certain increase of the content of free amino acids, although very small in comparison with the previous stage, is observed at the end of drying as well (Table 4c).

Among the examined neck samples, subjected to the same production procedure but injected with different combinations of selected cultures, there were observed considerable differences regarding the formed amino acids.

The highest content namely increase of free amino acids was found in samples of "bundiolla" produced with the selected culture combination C, being somewhat lower in samples produced with the combination B (Tables 4a, 4b and 4c).

The lowest content of free amino acids, after drying for 30 and 36 days, was observed in control samples (Tables 4b and 4c). However, immediately after curing (Table 4a), the lowest content of free amino acids was established in samples injected with the culture combination A.

3. Organoleptical examination of dry neck injected with selected culture combinations.- From Tables 5 and 6, as well as from Figure 2, it can be seen that neck samples injected with the selected culture combination B, and to a smaller extent neck samples injected with the combination C, show the most desirable organoleptical properties; taste, odour, colour and overall acceptability of these neck samples essentially surpass those of other samples. Organoleptical properties of samples injected with the culture combination A almost do not differ from those of control samples; however, organoleptical properties of samples injected with the culture combination D are even worse than those of control samples.

B. Examinations of dry bacon

Experimental production of dry bacon by applying selected culture combinations

As already mentioned, the selected microorganisms and their combinations show desirable fat changes on the medium prepared from fatty tissue. Therefore, simultaneously with "bunciolla", dry bacon was experimentally produced too.

Pieces of bacon, weighing 1000 g. on average, were cured with dry curing mixture of the same composition as in experimental production of dry pork neck. "Salted" pieces were injected with 2% of suspensions of culture combinations A, B, C and D, by means of long, perforated needles. The control group was injected with the corresponding quantity of 0.5% sugar solution.

After curing at 2 - 4°C for 14 days, bacon was dried in the "Trovaglini" clima chamber at 12 - 15°C and at relative humidity of 75 - 85%, for 36 days.

1. Bacteriological examination of bacon injected with selected culture combinations, during its curing and drying.- Figure 3 shows the results of total bacteria count, as well as the counts of lipolytic and saccharolytic microorganisms in bacon, after its curing for 14 days and after drying for 10 and 36 days.

As seen from Figure 3, total bacteria count and counts of lipolytic and saccharolytic bacteria, in all samples of bacon injected with selected culture combinations, range mainly within the limits of 10^7 and 10^8 in 1 g. The only exception

is a considerably lower count of lipolytic organisms in samples injected with the culture combination A, namely a very low count of organisms with saccharolytic properties in samples injected with the culture combination C. The control group shows considerably lower counts of all examined microorganisms.

Total count of bacteria, as well as their lipolytic activity, is decreased after curing for 14 days. The exception are again the samples marked as A and C, where the count of microorganisms with lipolytic, namely saccharolytic properties, is increased. In control samples, there is not any more essential change in relation to the previous stage.

In finished products, namely in bacon after its drying for 36 days, total bacteria count is equal to values obtained in the previous stage or slightly decreased. The same can be stated for their lipolytic and saccharolytic activities. As for control samples, total count of bacteria and their biochemical activities show very small oscillations in this stage.

2. Chemical examinations of dry bacon injected with selected culture combinations, during its curing and drying.-The results of some chemical examinations of bacon injected with selected culture combinations, during its curing and drying, are presented in Tables 7, 8 and 9.

Peroxide value of fat extracted from bacon is increased from stage to stage. However, while in control samples and in bacon injected with culture combinations C and D, after their drying for 36 days, i.e. in finished products, peroxide value reaches about 4, in samples produced with culture combinations A and B, this value is increased to over 11, namely 8.

The content of free fatty acids in bacon is also constantly increased till the 10th day of drying, the increase being slight in the majority of samples. In finished products, i.e. after the completion of drying, the content of free fatty acids in bacon produced with culture combinations A, B and C is over 1%. According to expectations, the contents of nitrite and salt are gradually increased, although unevenly in different bacon samples; the content of nitrate shows a reverse trend during all stages.

3. Organoleptical examination of dry bacon injected with selected culture combinations.- The results of organoleptical evaluation performed by 5 judges according to the score system with 9 points, are presented in Tables 10 and 11.

As seen from Tables 10 and 11, the samples of bacon produced with the culture combination C are most highly evaluated regarding their organoleptical properties. The difference is specially expressive in the evaluation of thier taste, in comparison with both the controls and other experimentally produced dry bacon samples. The same samples (C) were judged as acceptable for colour (Figure 4) and very acceptable for overall acceptability. The above mentioned statements, but to somewhat lower extent, apply also to dry bacon produced with the culture combination D, the colour of which (referring to both muscular and fatty tissue) was judged as very acceptable. Organoleptical properties of dry bacon injected with culture combinations A and B are identical to, or only slightly different from, those of control samples.

C. Examinations of ham ("pršut")

1. Microflora of "pršut" during its drying and after the completion of drying

Total bacteria count and individual counts of halotolerant, saccharolytic, lipolytic and proteolytic bacteria were examined during drying - at intervals of 30 days, as well as after the completion of drying. The obtained results are presented in Figure 5. As seen from Figure 5a, the surface of ham is richer in microflora after drying than after curing. Total bacteria count is considerably increased after the first and the second month of curing, and afterwards it remains approximately the same till the end of drying - about 10^7 in 1 g. of meat. The same can be stated for their biochemical activities, if counts of lipolytic, saccharolytic and proteolytic bacteria are considered.

Microorganisms with saccharolytic, lipolytic and proteolytic properties were also found in deep interior layers of "pršut" during the whole period of drying, although in a very small number (Figures 5b and 5c).

2. Dominant microflora of "pršut" during its drying

Results obtained by the examination of microflora being dominant during the 6-month drying of "pršut" are presented in Figure 6.

More than 80-90 percents of microorganisms found on the surface of "pršut" during its drying and after the completion of drying are Gram-positive, catalase-positive micrococci. Only about

8 - 16 percents of microorganisms are yeasts. The small number of microorganisms found in deep interior layers of "pršut" represents micrococci.

3. Chemical examinations of "pršut" during its drying and after the completion of drying

Basic chemical indices of "pršut" during its drying and after the completion of drying are presented in Table 12.

As it could be expected, the content of water was gradually but constantly decreased during the process of drying. In finished product, i.e. after drying for 6 months, the content of water was 40 percents. The contents of protein, fat, ash and salt were increased. During curing, the content of nitrites was increased and already after drying for 30 days it was considerably decreased. Decrease of the nitrite content was continued even after 2-month drying. After drying for 2 months, as well after the completion of drying, the content of nitrites was very low - hardly about 1 mg%. It was similar with the content of nitrates, except that the obtained values were higher. After the completion of drying, "pršut" contained about 2.4 mg% of $NaNO_3$.

At the beginning of "pršut" drying, pH value did not show any more essential changes. After drying for 4 months, pH value was considerably changed, being about 5.87 in the finished product.

Significant changes of fatty tissue were observed during drying of "pršut". The peroxide value was constantly increased, from month to month, but in the finished product it was not more than 4.5. The same happened to the quantities of free fatty acids, amounting to about 8 percents in the finished "pršut".

The quantities of free amino acids were also examined during drying of "pršut". The obtained results are presented in Tables 13. and 14.

As seen from the presented results, the content of free amino acids was constantly increased, with lower deviations, up to the 180th day of drying. This increase was more expressive during the first period of aging, up to the 90th day of drying. The most noticeable increases were those of the contents of glutaminic acid, leucine and isoleucine, alanine, phenyl alanine, asparaginic acid, serine and valine. A certain decrease of the contents of histidine, proline, threonine, lysine, glycine and tryptophan was observed at the end of the examined drying period of "pršut".

DISCUSSION

Salting of large meat pieces - most frequently with sea salt - has been known for centuries. Already in IX centry, as recorded by Homer, ancient Greeks salted pork in order to prolonge its shelf life. Gabrius Apicis, Roman writer, described in details salting and smoking of ham in the time of Czar Dioclatian. Ferrugio Faelli (1911) thinks that the modern procedure of "pršut" salting in Europe traces its origin to a Holland fisherman, Wilhelm Benkelz, who lived in VI century. According to Jensen, even the word "bacon" - comprising salted, cured and smoked pork, originates from the Spanish word "bucan" - salted and smoked pork.

Production of cured meat pieces is a process where each previous stage causes changes in the subsequent stage. Consideration of one stage only does not contribute to the understanding of the essence of the production, since it cannot explain all the changes manifested as specific organoleptical characteristics of finished products. In the whole complex of happenings during dry curing and subsequent drying, it is almost impossible to consider separately only microflora and its influence on the changes occuring at that time. Therefore our discussion on the examination results referring mainly to the role and changes of microflora during long-term dry curing, encircles all stages - starting from the raw material up to the finished product.

Count and kinds of microorganisms in pork and bacon during their long-term dry curing.- There are many data in the literature on the count and kinds of microorganisms during curing and "aging" of meat products, such as "pršut" and bacon. The same cannot

be said for "bundiolla", namely meat products consisting of smaller pieces of meat, for which the literature data can hardly be found. Due to many differences in the way of production, as well as due to different technique of bacteriological examinations used by various authors, the results of already published works referring to the significance of microflora during long-term curing of meat, can hardly be evaluated, compared and used. Different interpretation of simular or even identical results by various authors makes more difficult the comprehension of essential particularities. Nevertheless, the role played in the changes occuring during curing of meat is attributed to microflora by the majority of research workers. These changes influence the formation of desirable organoleptical properties of finished products. As for the findings of bacteria count, they differ to a great extent. These differences are due to already emphasized differences in curing methods: dry, dry-wet and wet curing. These differences should be emphasized, because the values obtained by individual authors range from 1000 to 100,000.000 in 1 ccm of curing brine, namely 1 g. of cured meat. It is the first datum explaining why there is not an uniform opinion of the role of microorganisms in physico- and biochemical changes occuring during curing of meat.

The second important datum refers to microorganism kinds stated as microflora of curing brine and meat during curing. A group of authors directed their examination exclusively towards the identification and classification of halopsychrophilic bacteria, which - as dominant flora - are presumed to play a significant role in long-term curing at low temperatures. Other authors approach the examinations without any theoretical prejudices. On the basis of comprehensive examinations, numerous kind of bacteria, and from recently yeasts as well, are cited as microflora of curing brine and meat. According to the results of

such examinations, the following kinds of bacteria are most frequently present in the curing brine: micrococci, streptococci, lactobacilli, achromobacter, pediococci, vibrio, coliform, artrobacter, staphylococci, phlavobacterium, and then unidentified halophilic and psychrophilic bacteria, as well as moulds and yeasts.

It can positively be declared that in the literature there are not works dealing with mutual relation of microorganism kinds found in curing brine or cured meat, nor the data showing which kinds of microorganisms are the characteristic and dominant ones in a particular curing procedure.

On purpose to establish more precisely the participation of microflora in biochemical changes during meat curing, the subject of examinations carried out in the course of a few last years was the enzymic activity of some microorganism kinds isolated from curing brine or meat. Although it has not still been established which biochemical activities - and to which extent - are necessary for desirable development of changes during curing, it seems that such bacteriological examinations will show more clearly which microorganism kinds and counts can be considered as useful ones. The examinations of biochemical changes in cured meat carried out so far, have given certain data - although not defined in details - on the carriers of taste and odour of cured meat. These examinations could be used on occasion of studying the enzymic activity of microorganisms, as an indication for finding out their positive role in the formation of characteristic organoleptical properties of cured and smoked meat pieces. Practical application of so-called "starter" cultures to other kinds of meat products has shown that the activity of "starter" cultures is not dependent on their number only, but even more on their biochemical activity as well as on their relation to other microorganism kinds being present at the same time.

On the basis of the examination results obtained untill now and known to us, it can be stated that during curing microorganisms take part in the changes of carbohydratec, fat proteins and curing salts. The question is which kinds of microorganisms, and in which number and relation, manifest the most expressive activity.

By examining various parts and pieces of pork, differing essentially one from another regarding their microstructure and relation of muscular and fatty tissues, we defined the procedure of dry curing, the composition of dry curing mixture and microclimatic conditions. For the curing procedure defined in such way, as well as for the drying procedure defined later on in the same way, we established the total count and dominant kinds of microorganisms in two successive lots and at two different meat packing plants. At the same time, we examined the biochemical activity of dominant microflora which can be of significance for the changes occuring during dry curing of meat.

The obtained results point to different rates of penetration of microorganisms into meat during its during. In large meat pieces, such as ham, having a larger part of surface covered with skin and subcutaneous fatty tissue, the microorganisms are found only in the periphery layer of the medial side of ham, whereas in smaller pieces, such as neck and bacon, the microorganisms penetrate into deep interior layers. However, a common property of all examined pieces is that the count of microorganisms is gradually but constantly increased from the beginning to the end of dry curing process. There are differences in total bacteria count, but they are dependent on the initial count, i.e. count in raw material, and only to a smaller extent on the curing procedure. Although the temperature of curing ranged from 0 to 4°C, the microorganism

medial surface layer of ham. On the basis of these results, it can be stated that the increase of microorganism count, from about 10^3 - 10^4 in 1 g. of raw meat to about 10^6 in 1 g. of cured meat, is characteristic for the examined dry curing procedure, lasting 30 days on average.

By the applied examination technique it has been established that the majority of microorganisms being present during dry curing has several significant biochemical characteristics. Microorganisms adapt themselves to the medium containing a higher quantity of common salt. At relatively low temperatures ranging from 0 to 4°C, microorganisms are able to reduce nitrites and to decompose sugar and fatty tissue. However, the count of microorganisms with proteolytic properties is very low.

Dominant microflora of pork during its dry curing. - Group identification of dominant microflora during dry curing has shown that in meat of neck and bacon, as well as in the surface layer of ham, Gram-positive cocci are present in the highest number, being followed by Gram-positive bacilli; yeasts are also present but in a lower number.

Regardless of all variations during examinations, it has been established with certainty that more that 60% and even up to 75% of all microorganisms present during dry curing of neck, ham and bacon, are Gram-positive, catalase-positive cocci. They represent characteristic and dominant microflora of the examined dry curing procedure. Other microorganism kind (catalase-negative and positive bacilli, yeasts, unidentified halotolerant and psychrophilic bacteria) are accidental followers of the process. It is probable that their mutual relation depends on the initial count in raw material and on their capability to adapt themselves to the conditions of the examined

curing procedure. Nevertheless, some of them such as Gram--positive catalase-negative bacilli and yeasts, possess biochemical properties which can cause desirable changes in meat during its curing. For that reason, although present in a low percentage - 10 to 20%, their role must not be neglected.

Biochemical activity of selected microorganism kinds on natural substrates - meat and fatty tissue.- It has been emphasized in the literature that the properties of microorganisms, obtained on nutritive synthetic media in laboratory conditions, are not manifested in the same form and to the same extent when examined in meat products. On purpose to select active microorganisms being potentially useful in both meat and fatty tissue, we applied our own procedure. The results obtained by applying our own procedure indicate that - only a certain number of microorganisms, which showed a rich enzymic activity in the laboratory test, retains this property on occasion of growth on both fatty tissue and meat. Therefore it can be stated that the applied procedure of culture selection on media prepared from fatty tissue, namely meat, represents a good method for finding out the strains which can successfully be used for the acceleration of a defined method of curing, and even drying ana aging.

Conditions for practical application of selected microorganism cultures during dry curing of pork.- Numerous literature data on dry sausages are the best proof that it is not simple to apply "starter" cultures to meat products in the practice. Although the first patented "starter" cultures trace back to 1902, their application to dry sausages has not proved to be of more practical importance so far. In the course of a few last years, new procedures of bacteria growth for practical application in meat industry were examined in various countries

and in various products (Dževizov, Petrov, Mihajlova). Instead of the data on bacteria in frozen or freeze-dried form, the data on the application of substrates in which the bacteria were previously grown are more and more found in the literature. These data are based on theoretical considerations and partially on practical results. The results of our examinations show that the effect of the application of microorganisms depends on the procedure by which they were prepared prior to injection into meat pieces. Having in mind that curing is performed at low temperatures which retard the growth and biochemical activity of microorganisms even when they were adapted to psychrophilic conditions, the way of culture preparation for their injection into meat - already difined in our work - contributed to their efficient activity during curing. The sugar solution in which the microorganisms were grown prior to their injection into meat, and temperature of 0 - 4°C at which they were grown, contribute to the adaptation of microorganisms to new conditions of growth (meat), probably by activating their enzymes. Alive cells in the suspension, supported probably by enzymes formed or activated already in the sugar solution, express their activity immediately after injection. The phase of adaptation lasts a very short period of time.

Our results show that besides the choice of adequate microorganism kinds, the choice of an adequate method of preparation of these microorganisms prior to their introduction into meat pieces, is also - perhaps even more - significant. Although we could not check our presumption due to the lack of time, it seems to us that probably a complex of extracellular enzymes is present in the suspension of cultures being previously kept in frigidaire for 5 - 7 days. The enzymes act immediately after the injection of cultures into meat, and later, on

during curing, the activity started by enzymes secreted in the medium in which the bacteria were grown is continued by the present bacteria (in meat) secreting new enzymes as a result of their life functions in the new medium to which they already have been adapted. On the basis of up to now obtained results, it can be presumed, with enough probability, that the use of isolated bacterial enzymes in the curing of meat pieces, could be a further trend of the examinations. To prove this presumption, numerous examinations are necessary.

Experimental production of "bundiolla" and bacon. - As far as we know, the data on practical application of microorganisms during curing of "bundiolla" and bacon cannot be found in the literature. Neither are there attempts of previous treatment of hams for "pršut" with microorganisms or products of their metabolism. One of the reasons for that is certainly the long-term way of production necessary for the comprehension of all desirable or undesirable results of the activity of the used selected culture or cultures. The small number of examinations was therefore limited to laboratory conditions. Since we succeeded in reducing the time of the laboratory part of examinations by using a specific procedure for the selection of bacterial cultures, we produced "bundiolla" and bacon in practical conditions by applying selected microorganisms. Hams for "pršut" could not be examined in the same way due to the lack of time - the results could be obtained only after drying for 8 - 10 months.

The results obtained by the production of "bundiolla" and bacon in the practice show that the time of curing can considerably be shortened, from 30 to 10 - 12 days, by applying the selected microorganisms combinations. In addition, certain culture combinations not only stabilize the specific and desirable properties of bacon and "bundiolla", but they also improve them to a considerable extent. As for the specific

taste and odour, changes of amino acids, namely of the content of free fatty acids, are also very significant for their formation.

Organoleptical examinations show that bacon produces with the combinations C (micrococcus 018 and yeast 059) has considerably better colour, taste and odour in relation to both control samples and other examined microorganism combinations. Bacon produced in such was has the lowest peroxide value (below 4) and, at the same time, a higher content of free fatty acids already after drying for 10 days. Further examinations, namely analysis of the composition of formed free fatty acids, and perhaps some other compounds being not the subject of this work, could probably explain in more details the role of applied microorganisms and their connection with compounds causing the improved quality of bacon.

According to organoleptical evaluation, "bundiolla" produced with the culture combination B (micrococcu 018 and Lactobacillus plantarum), and to a lower extent "bundiolla" injected with the culture combinations C (micrococcus 018 and yeast 059) essentially surpass the controls and all other experimental samples, regarding odour, taste, colour and overall acceptability.

"Bundiolla" produced with the culture combination C has the highest content of free amino acids. Somewhat lower content is observed in "bundiolla" samples marked with B. It is very significant that samples of "bundiolla" being organoleptically evaluated as the best ones, have different contents of individual amino acids, in relation to both control samples and other experimental samples.

Our results confirm the already presented point of view that the problem of the production of "bundiolla", bacon and "pršut" should be comprehensively considered. The examination of microflora only, or biochemical changes only, cannot explain the complex happenings. By simultaneous examination of biological, physical and chemical changes during experimental production of bacon and "bundiolla", starting from the raw materioa up to the finished product, we succeeded to some extent in realizing more clearly the role of microorganisms during curing and "aging" of these products.

It seems to us that, besides the concerete results representing a contribution to better knowledge of microflora during curing of pork, our four-year examinations have also a deeper significance. By the development of a technique adapted to quicker obtainment of data on the properties of microflora and its biochemical activities in relation to meat and fatty tissue, and by establishing the data on the procedure of microorganism preparation for the use in meat products, as well as by simultaneous following of changes in basic components of meat and fatty tissue, we established our own system of strudy within this field of meat science. The application of cultures isolated during a definite procedure of production, selected by a simple technique and adapted to the conditions of the defined product, gave good results being reflected not only in the improvement of desirable organoleptical properties of the product but also in the reduction of the production time. This is of special significance for meat industry from the standpoint of both technology and economy.

CONCLUSIONS

1) Procedures of dry curing of neck (for "bundicl1a"), bacon, and ham (for "pršut"), lasting 20 - 30 days, have a common characteristic regarding the total bacteria count. At the end of curing, after gradual but constant increase, the bacteria count was about 10^6 in 1 g. of cured meat.

2) In all stages and at the end of dry curing of neck, bacon and ham, micrococci were the dominant microflora, representing about 60 - 70 percents of all microorganisms. Other microorganisms were Gram-positive, catalase-negative bacilli and yeasts (20 - 30%), being in a mutually changeable relation.

3) At low temperatures ranging from 0 to 4^oC, a high percentage of dominant microorganisms is able to reduce nitrites and to decompose sugar and fatty tissue.

4) On occasion of growth on fatty tissue, the selected microorganisms isolated during dry curing contribute to higher formation of free fatty acids and other compounds appearing as a result of fat hydrolysis.

5) The selected microorganisms and their combinations express an useful effect on meat during its dry curing, if they were previously prepared by being kept - suspended in 0.5% sugar solution - at 0 - 4^oC for 5 - 7 days.

6) When applied in the production of bacon, the selected microorganisms in the combinations C (micrococcus 013 and undetermined yeast 059) contribute to the reduction of curing time, from 30 to 12 days.

7) The selected culture combination B (micrococcus 018 and Lactobacillus plantarum), and to somewhat lower extent the culture combination C (micrococcus 018 and yeast 059), when injected into pork neck intended for "bundiolla" production, reduce the time of curing to a considerable extent. The products obtained in this way show considerably better taste, odour and colour than the controls.

8) In hams being dry cured for the production of "pršut", microorganisms are present (in already mentioned number) only in the medial surface layer. During further curing, as well as during six-month drying of "pršut", the bacteria count in deep-interior layers and in lateral surface layer ranges from 10 to 1000 in 1 g. of meat.

9) Our results, checked in practice and summarized in the conclusions, confirm the possibility of essential changes in the production process of cured pork pieces. The application of selected cultures prepared by being grown in the sugar solution, results in essential reduction of the production process and in the improvement of organoleptical properties of cured and dried pork pieces, in relation to the usual production process.

Basic chemical indices of pork neck injected with selected culture combinations, after the completion of curing

Table 1.

	Neck samples					
	Raw meat	Cured controls	A	B	C	D
Water, %	66,3	65,90	66,80	66,10	63,80	65,31
Fat, %	13,50	13,15	19,70	10,40	13,75	12,62
Protein, %	19,30	16,30	14,90	18,35	17,70	17,16
NaCl, %	0,07	3,71	4,08	4,29	4,04	3,93
$NaNO_2$, mg%	0,00	3,77	4,00	4,50	3,00	5,00
$NaNO_3$, mg%	0,00	1,50	2,83	3,20	2,14	3,93
pH	6,20	6,20	6,50	6,55	6,50	6,05
PV	0,00	0,43	0,06	0,00	0,14	0,37
FFA, %	0,37	0,24	0,27	0,35	0,39	0,45

PV = Peroxide value

FFA = Free fatty acids

Basic chemical indices of pork neck injected with selected culture combinations, after drying for 20 days

Table 2.

	Neck samples:				
	Control	A	B	C	D
Water, %	45,40	44,80	46,10	45,80	46,70
Ash, %	25,35	19,80	21,10	20,80	19,50
Protein, %	23,10	27,75	25,05	27,60	27,[illegible]
NaCl, %	4,95	6,59	5,93	5,38	5,[illegible]
$NaNO_3$, mg%	5,75	6,40	5,80	5,50	6,70
$NaNO_2$, mg%	1,40	2,82	2,70	1,35	3,75
pH	5,85	6,28	5,35	6,20	5,90
PV	1,52	3,55	1,10	3,50	3,25
FFA, %	0,46	0,72	0,60	0,80	0,50

PV = Peroxide value

FFA = Free fatty acids

Basic chemical indices of dry pork neck produced experimentally by applying selected culture combinations

Table 3.

	Neck samples				
	Controls	A	B	C	D
Water, %	32,70	40,16	35,20	37,01	35,70
Fat, %	23,30	25,60	27,60	21,90	23,70
Protein, %	33,40	31,90	29,40	30,10	34,20
NaCl, %	5,00	6,74	6,68	6,30	4,80
$NaNO_2$, mg%	4,50	5,70	6,43	6,30	6,00
$NaNO_3$, mg%	1,23	2,63	2,45	1,30	3,31
pH	5,80	6,10	5,80	6,20	5,80
PV	4,29	4,77	3,63	4,15	3,66
FFA, %	0,81	2,04	0,90	2,12	1,22

PV = Peroxide value

FFA = Free fatty acids

Content of free amino acids in "Bundiolla" injected with selected culture combinations, after the completion of curing (µmole/g. of sample)*

Table 4a

Amino acids	Raw pork neck	Cured pork neck injected with culture combinations				Controls
		A	B	C	D	
Leucine + isoleucine	0,106	6,258	5,583	7,429	6,773	4,598
Phenyl alanine	0,121	2,151	2,145	2,163	2,175	2,141
Lysine	1,089	1,710	2,240	1,647	1,231	1,233
Tryptophan	0,105	0,488	0,374	0,994	0,982	0,973
Methionine	0,092	1,100	1,221	1,467	1,432	1,145
Valine	0,540	1,820	1,970	2,605	1,582	1,630
Serine	4,165	9,420	9,890	11,435	9,810	9,990
Proline + threonine**	0,588	0,778	4,310	4,390	4,390	4,200
Glutaminic acid	0,938	2,865	5,110	4,148	2,460	2,364
Glycine	0,966	1,881	2,184	2,330	2,330	1,729
Alanine	0,432	2,340	3,517	3,200	2,658	2,363
Asparaginic acid + arginine***	0,451	2,510	2,865	2,165	2,315	2,296
Histidine	0,396	2,900	2,968	2,902	2,935	3,163
Tyrosine	0,152	0,900	1,210	1,912	1,916	1,032
Total content:	10,141	37,130	46,993	48,787	42,989	39,353

* Mean value of three samples

** Proline + threonine - expressed as proline

*** Asparaginic acid + arginine - expressed as arginine

Content of free amino acids in "Bundiolla" injected with selected culture combinations, after drying for 20 days

(µmole/g. of sample)*

Table 4b

ino acids	"Bundiolla" injected with culture combinations (after drying for 20 days)				Controls
	A	B	C	D	
ine + isoleucine	13,170	14,165	14,570	13,950	11,710
ylalanine	3,118	4,825	4,835	4,845	3,232
ne	6,080	5,652	6,607	4,980	3,495
tophan	1,622	1,722	1,793	1,485	1,360
onine	4,700	4,327	6,710	5,703	4,208
ine	2,880	2,497	3,387	2,110	2,931
ine	15,100	16,050	14,820	9,890	13,793
ine + threonine**	6,200	5,180	6,543	6,380	6,141
minic acid	10,460	9,124	8,754	8,510	8,442
ine	2,983	3,470	3,705	3,860	2,965
	2,920	3,980	3,950	2,643	3,023
ginic acid + ine***	4,840	5,938	5,905	4,676	4,608
dine	3,870	4,857	4,340	4,280	3,960
	1,710	1,725	1,962	2,175	1,644
content:	79,653	83,512	87,871	75,517	72,498

ge value of three samples

ine + threonine - expressed as proline

araginic acid + arginine - expressed as arginine

Content of free amino acids in "Bundiolla" produced experimentally by applying selected culture combinations

(γ mole/g. of sample)*

Table 4c

Amino acids	"Bundiolla" injected with culture combinations (after drying for 36 days)				Controls
	A	B	C	D	
Leucine + isoleucine	12,845	13,710	14,560	14,005	11,828
Phenylalanine	3,687	5,420	5,335	5,246	4,420
Valine	5,680	5,830	6,070	5,040	4,083
Tryptophan	1,390	1,682	1,654	1,422	1,328
Methionine	5,020	4,367	4,672	4,705	4,700
Glycine	2,050	2,570	2,525	2,110	2,382
Proline + threonine**	15,890	15,690	15,330	13,160	11,260
Glutaminic acid	6,083	6,385	6,880	6,257	6,740
Serine	11,970	11,620	12,820	12,865	11,120
Alanine	3,260	3,470	3,632	4,335	3,162
Asparaginic acid + Arginine***	3,027	3,832	4,020	3,343	2,982
Histidine	4,570	5,880	5,747	5,905	5,674
Lysine	3,995	4,740	4,265	4,182	3,650
Tyrosine	1,974	1,838	2,172	1,975	1,717
Total content:	81,441	87,034	89,682	84,550	75,042

* average value of three samples

** proline + threonine - expressed as proline

*** asparaginic acid + arginine - expressed as arginine

Organoleptical evaluation of experimentally produced dry pork neck

Table 5.

Dry neck samples	Number of scores given by 5 juages for Taste	Odour	Colour	Overall acceptability	Total number of scores
Controls	29	34	33	32	128
A	29	31	34	33	125
B	35	36	38	36	145
C	31	35	40	34	140
D	21	25	33	26	105

Place order obtained by organoleptical evaluation of experimentally produced dry pork neck

Table 6.

Evaluated properties	Place order of samples				
	1	2	3	4	5
Taste	B	C	K	A	D
Odour	B	C	A	K	D
Colour	B	C	A	K	D
Overall acceptability	B	C	A	K	D

Basic chemical indices of bacon injected with selected culture combinations, after curing for 14 days

Table 7.

Bacon samples	Peroxide value	Free fatty acids	$NaNO_2$ mg%	$NaNO_3$ mg%	NaCl %
A	2,34	0,26	2,37	2,80	1,52
B	1,30	0,30	2,50	3,00	2,06
C	1,03	0,30	0,25	2,40	2,25
D	1,88	0,29	1,50	2,18	2,68
Controls	0,66	0,25	1,25	1,70	2,20

Basic chemical indices of bacon injected with selected culture combinations, after drying for 10 days

Table 8.

Bacon samples	Peroxide value	Free fatty acids	$NaNO_2$ mg%	$NaNO_3$ mg%	NaCl %
A	3,38	0,84	3,10	1,80	3,50
B	4,83	0,73	3,73	2,40	3,78
C	2,25	1,12	1,57	1,78	3,55
D	4,17	0,86	4,70	1,50	3,60
Controls	2,34	0,66	4,20	1,30	3,10

Basic chemical indices of dry bacon produced experimentally by applying selected culture combinations

Table 9.

Dry bacon samples	Peroxide value	Free fatty acids	$NaNO_2$ mg%	$NaNC_3$ mg%	NaCl %
A	11,65	1,99	3,40	2,06	4,68
B	8,44	1,06	4,50	2,19	3,00
C	3,79	1,21	2,40	1,60	4,38
D	4,12	0,95	4,50	0,70	4,16
Controls	4,73	0,88	4,60	1,50	3,90

Organoleptical evaluation of experimentally produced dry bacon

Table 10.

Dry bacon	Number of scores given by 5 judges for Taste	Odour	Colour	Overall acceptability	Total number of scores
Control	13	24	37	17	91
A	22	24	30	23	99
B	23	24	29	27	103
C	36	29	35	35	135
D	25	26	38	25	114

Place order obtained by organoleptical evaluation of experimentally produced dry bacon

Table 11.

Evaluated properties	Place order of samples 1	2	3	4	5
Taste	C	D	B	A	K
Odour	C	D	B, A, K	-	-
Colour	D	K	C	A	B
Overall acceptability	C	B	D	A	K

K = controls

Basic chemical indices of ham[a] during its curing and drying

Table 12.

Ham sample	Water %	Protein %	Fat %	Ash %	NaCl %	$NaNO_2$ mg%	$NaNO_3$ mg%	pH	Peroxide value	Free fatty acids
w ham	69,91	18,75	10,31	ø	ø	ø	ø	6,05	ø	0,15
n after curing r 30 days	62,50	20,55	12,25	4,94	3,65	6,35	4,20	6,39	0,57	0,37
"But" after ing for onth	62,00	23,07	9,77	5,30	3,79	4,16	3,27	6,27	3,87	0,87
onths	54,12	24,55	15,32	6,18	4,58	2,73	2,93	6,28	3,04	1,02
onths	50,01	25,00	18,32	7,20	5,60	1,48	1,28	6,10	2,94	2,87
nths	47,82	24,35	21,85	7,18	6,22	1,15	2,37	5,50	3,21	4,37
nths	45,30	24,20	23,45	6,90	6,69	1,59	2,54	5,70	3,30	5,15
shed product	41,35	26,25	24,60	7,73	6,20	1,12	2,40	5,87	4,45	7,96

rage value of three samples

Content of free amino acids in "pršut", during its curing and drying

(μmole/g. of sample)[4]

Table 13.

Amino acids	Ham		"Pršut" after drying					
	Raw	After curing for 30 days	1 month	2 months	3 months	4 months	5 months	6 months
Leucine + isoleucine	1,26	9,12	14,50	20,40	28,70	35,70	39,50	47,30
Phenyl-alanine	0,17	2,96	4,21	8,05	11,20	10,89	16,22	21,70
Valine	1,11	4,10	6,23	12,74	17,20	23,16	20,75	25,10
Tryptophan	0,25	1,52	2,39	4,21	6,08	7,38	7,92	8,52
Methionine	0,11	1,83	3,79	6,02	8,12	11,86	9,64	11,58
Tyrosine	0,65	1,45	2,40	4,49	5,60	5,57	7,20	10,52
Alanine	3,58	11,27	16,44	24,75	31,76	38,20	43,30	49,60
Proline[44] + threonine	1,02	7,08	11,27	19,20	27,52	32,35	26,05	27,35
Glutamic acid	1,66	6,97	9,68	18,70	28,35	34,60	45,50	58,20
Glycine	1,25	3,81	5,09	10,56	16,86	20,40	17,20	17,32
Serine	0,60	3,04	4,88	9,16	12,12	15,85	17,67	22,00
Aspartic acid + arginine[444]	0,58	5,08	5,61	9,00	14,37	18,80	20,65	25,80
Histidine	0,52	3,58	4,51	8,41	13,93	16,96	16,20	14,30
Lysine	0,17	0,27	0,50	0,91	1,20	1,05	0,96	1,35
Total content:	12,93	62,04	91,50	156,60	223,01	272,77	288,76	340,64

[4]Average value of three samples

[44]Proline + threonine - expressed as proline

[444]Asparaginic acid + arginine - expressed as arginine

Content of free amino acids in "pršut", during its curing and drying

(μmole/g. of dry substance)*

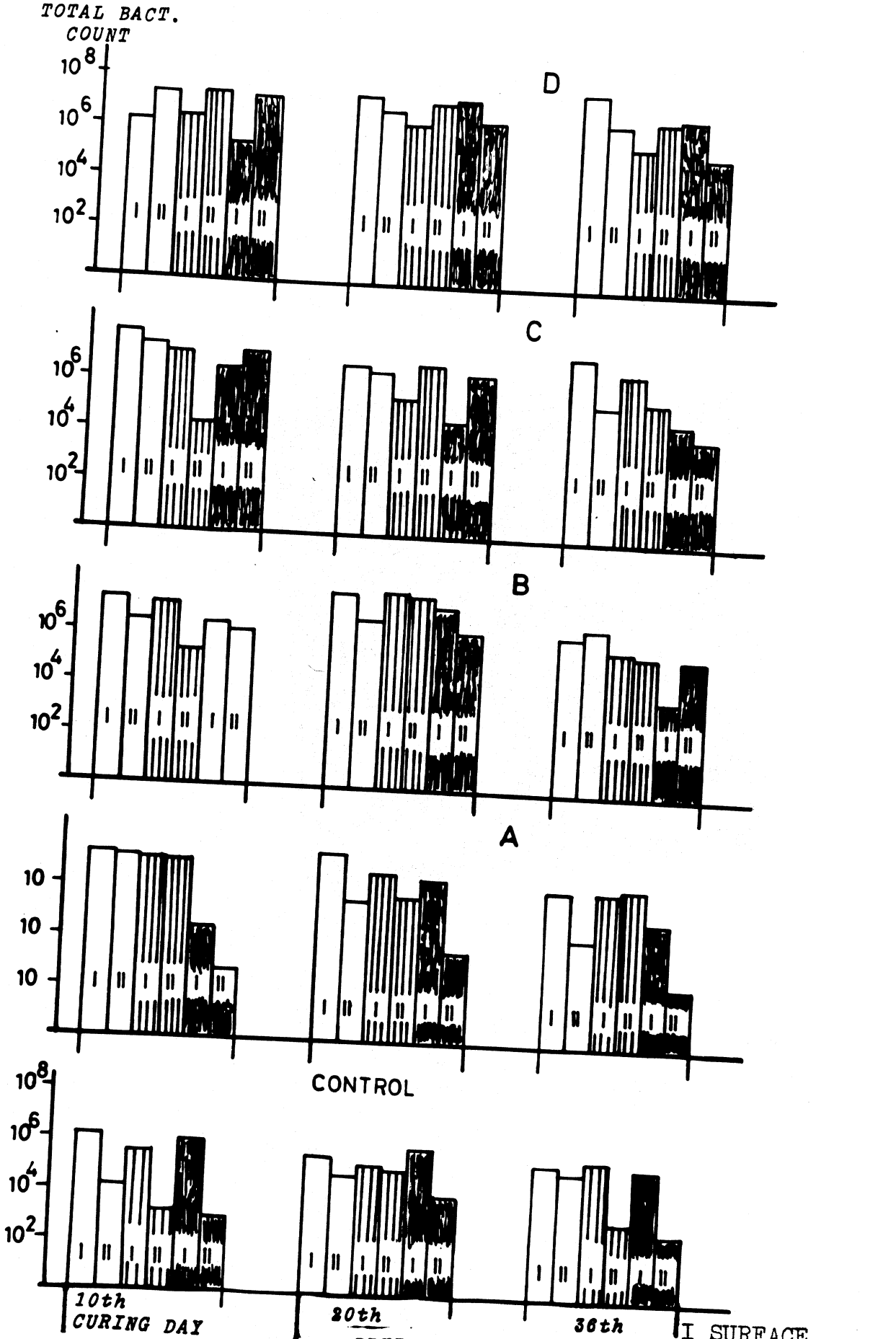
Table 14.

Amino acids	Ham		"Pršut" after drying					
	Raw	After curing for 30 days	1 month	2 months	3 months	4 months	5 months	6 months
Leucine + isoleucine	3,88	24,80	39,10	43,30	58,09	68,90	72,60	78,17
Phenyl-alanine	0,52	7,93	11,35	17,06	22,65	20,31	29,80	35,91
Valine	3,51	11,15	16,80	27,08	34,82	43,17	38,10	41,55
Tryptophan	0,78	4,14	6,45	8,94	12,30	14,25	14,56	14,11
Methionine	0,33	4,96	10,22	12,78	16,42	22,90	17,72	19,18
Tyrosine	2,06	3,94	6,46	9,63	11,34	10,76	13,24	17,42
Alanine	10,96	30,60	44,30	52,50	64,27	73,80	79,52	82,12
Proline** + threonine	3,14	19,22	30,39	40,71	55,70	62,47	48,70	45,25
Glutamic acid	5,08	18,95	26,12	39,70	57,36	66,80	83,60	96,40
Glycine	3,82	10,35	13,73	22,40	34,10	39,40	31,60	28,66
Serine	1,83	8,26	13,17	19,43	24,68	30,59	32,45	36,41
Aspartic acid + arginine***	1,77	13,80	15,12	19,10	29,08	36,25	37,94	42,74
Histidine	1,59	9,74	12,14	17,85	28,20	32,76	30,35	23,50
Lysine	0,52	0,73	1,34	1,34	2,42	2,03	1,77	2,24
Total content:	39,79	168,57	246,69	331,82	451,43	524,39	531,95	563,66

*Average value of three samples

**Proline + threonine - expressed as proline

***Asparaginic acid + arginine - expressed as arginine


TOTAL BACT. COUNT
D
C
B
A
CONTROL
10th CURING DAY
20th
36th
DRYING TIME (DAYS)
I SURFACE
II DEEP INTERIOR AREA
TOTAL BACTERIA COUNT
SACCHAROLYTIC BACTERIA
LIPOLYTIC BACTERIA

1.

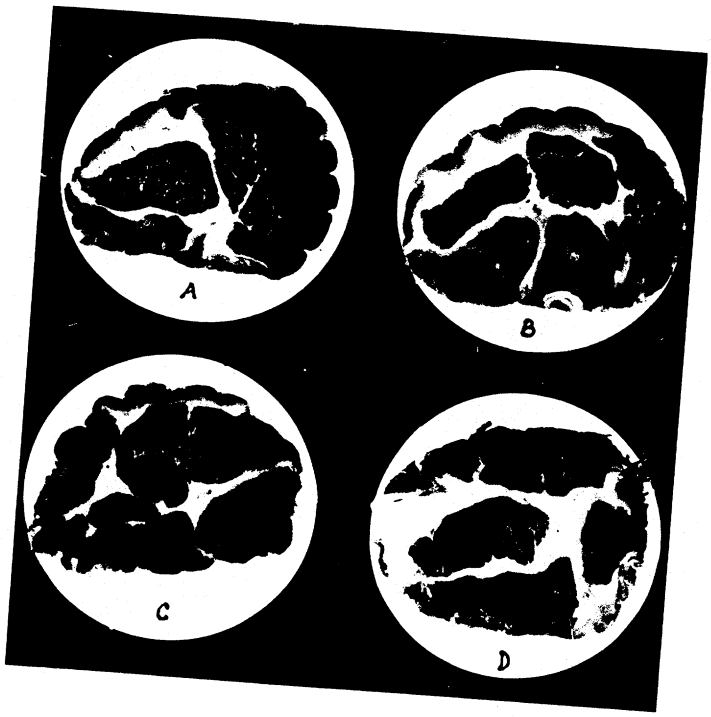


Fig. 2. Cut surface appearance of experimentally produced "Bundiolla"

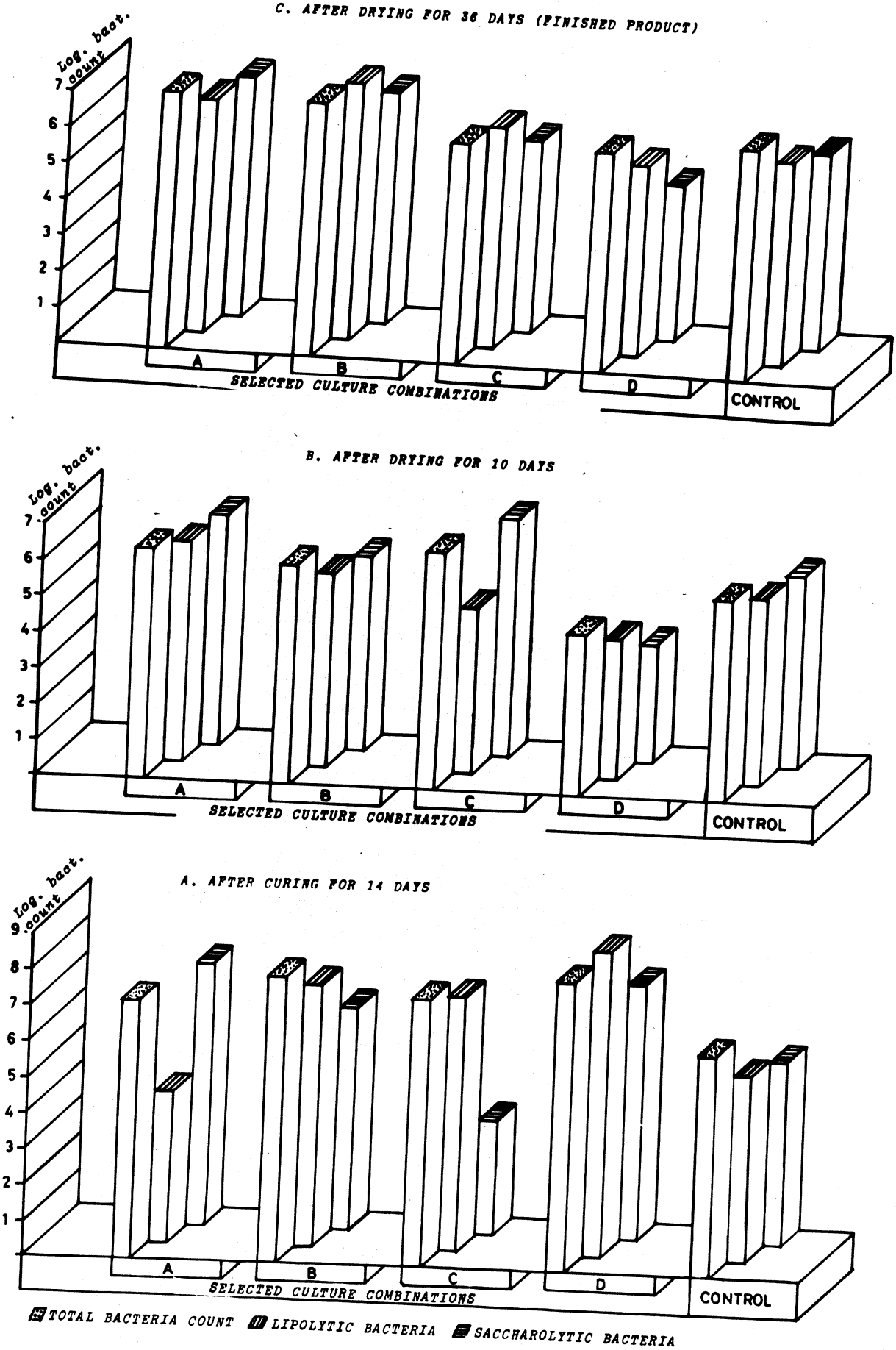


Fig. 3. MICROORGANISM COUNT OF BACON BEING INJECTED WITH SELECTED CULTURE COMBINATIONS

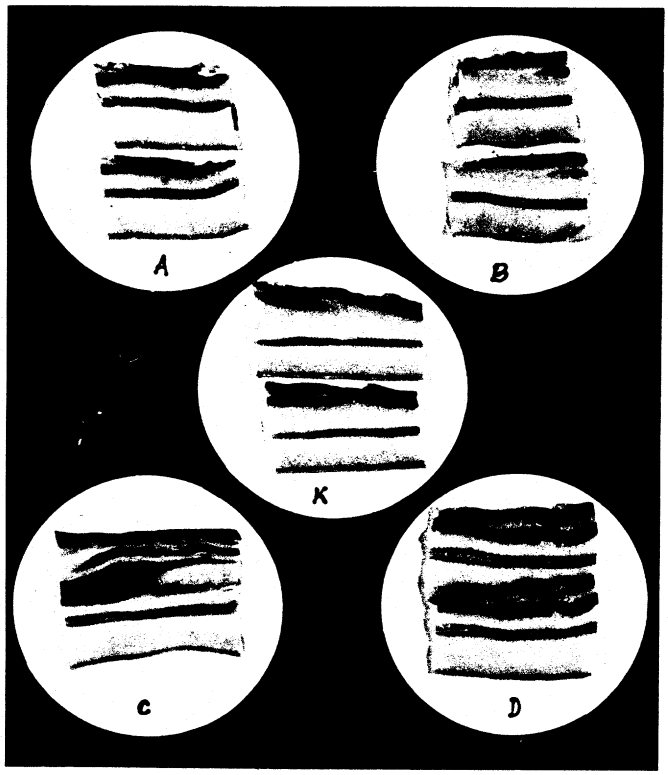


Fig. 4. Cut surface appearance of experimentally produced dry bacon

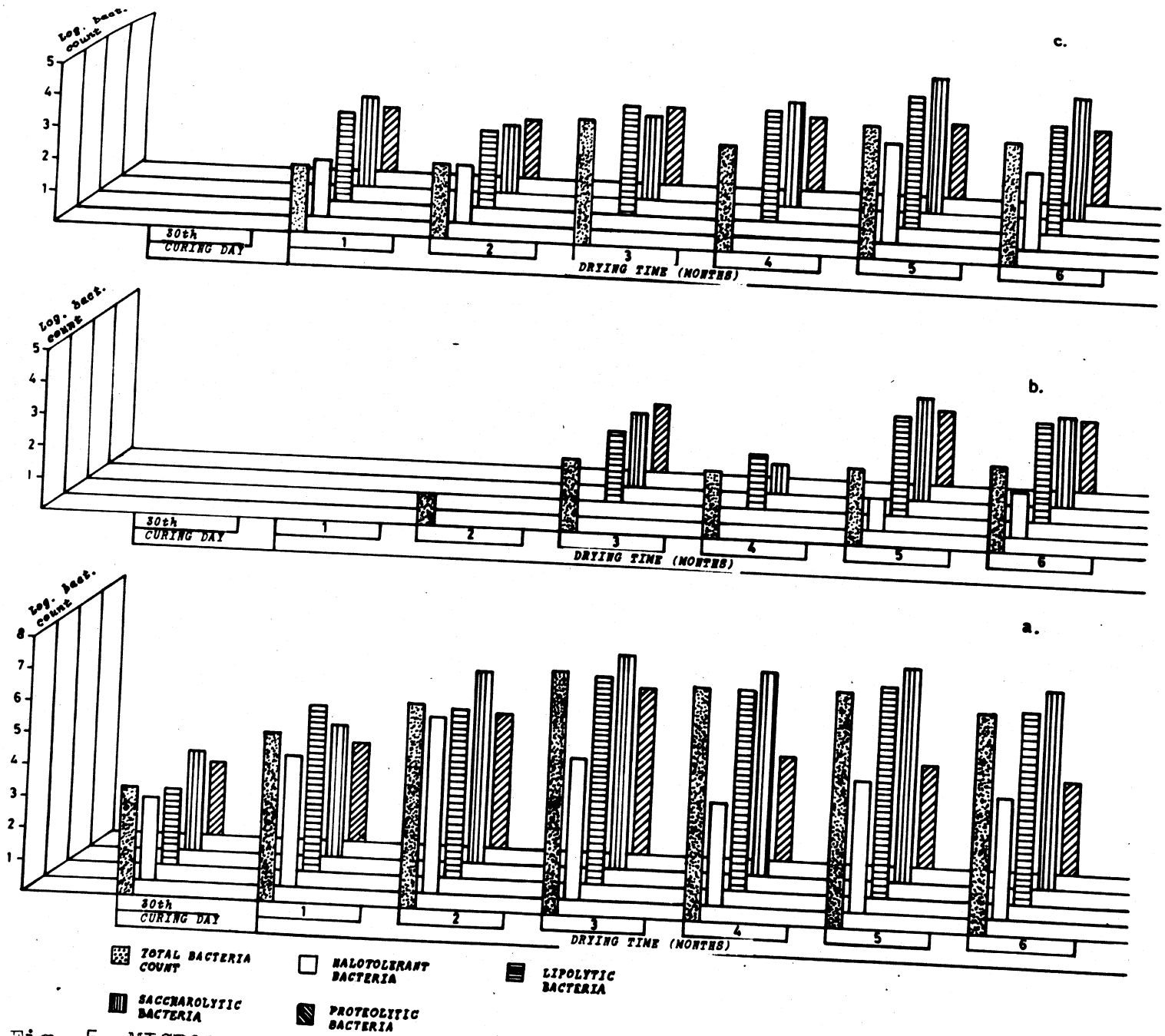


Fig. 5. MICROFLORA OF "PRŠUT" AFTER CURING AND IN 6-MONTH COURSE OF DRYING

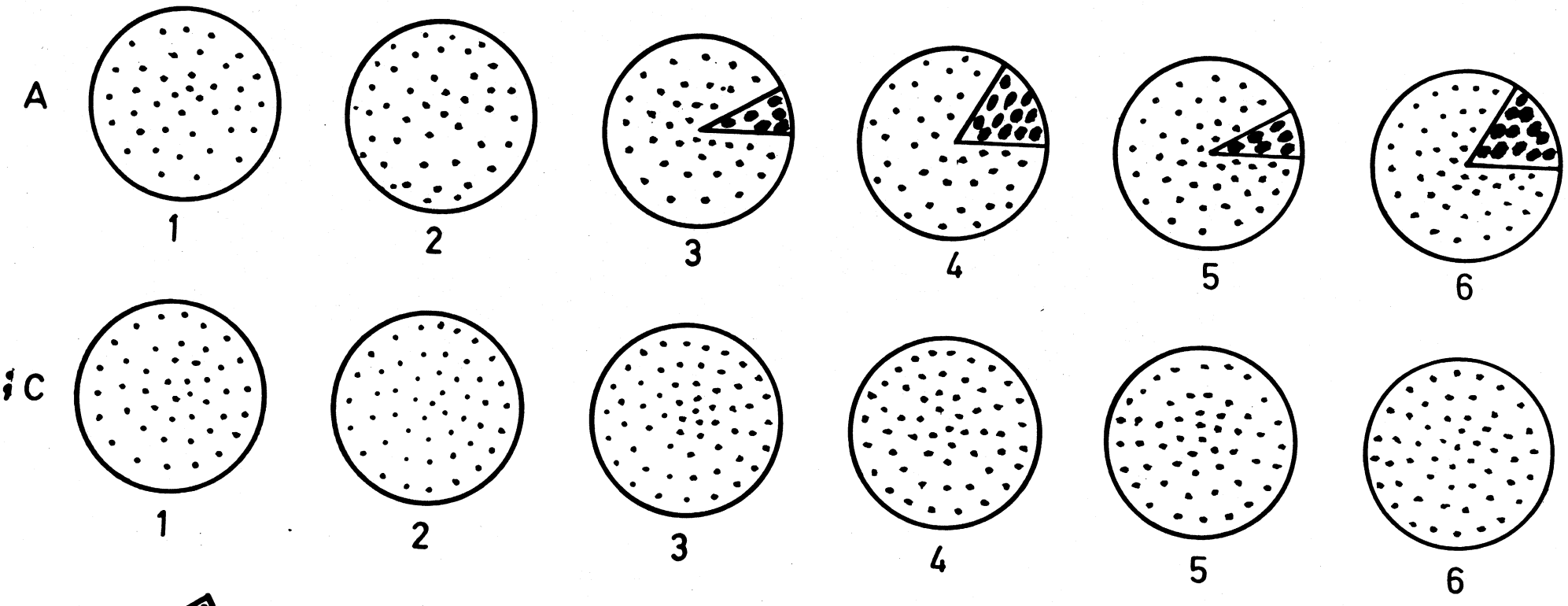


Fig. 6. DOMINANT MICROFLORA OF "PRŠUT" IN DRYING PERIOD